A translational approach to the genetics of renal disease

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Chapter 3

Possible renoprotection by vitamin D in chronic renal disease: beyond mineral metabolism

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Abstract

Vitamin D is typically viewed as a key player in the regulation of calcium and phosphate levels and the control of bone metabolism; however, growing evidence suggests that vitamin D deficiency may also have an important role in the progressive loss of renal function. Vitamin D deficiency is particularly frequent in patients with chronic kidney disease, in whom it is associated with increased mortality. Studies indicate that treatment with vitamin D analogues reduces proteinuria, suppresses the renin-angiotensin-aldosterone system (RAAS), and exerts anti-inflammatory and immunomodulatory effects. These pleiotropic effects render vitamin D a potentially interesting treatment modality for renoprotection in patients with chronic kidney disease. Whether vitamin D has clinically relevant renoprotective effects in addition to RAAS blockade is currently under investigation.

Introduction

Vitamin D has been recognized for decades as a key player in the control of bone metabolism through the regulation of calcium and phosphate homeostasis. This hormone regulates serum concentrations of calcium and phosphate by stimulating the absorption of these minerals in the intestine and their reabsorption by the kidney. Vitamin D deficiency results in increased circulating levels of parathyroid hormone (PTH), which has several disadvantageous effects on bone metabolism. PTH activates osteoblasts to stimulate the maturation of osteoclasts. Mature osteoclasts degrade the mineralized collagen matrix in bone, resulting in osteopenia, osteoporosis, and increased risk of fracture. Another important consequence of high circulating levels of PTH levels is phosphaturia, which can result in low levels of serum phosphate. Consequently, the calcium-phosphorus product decreases, which results in decreased mineralization of the collagen matrix and can lead to the development of rickets in children and osteomalacia in adults (1).

The decline in the incidence of rickets that was observed following the fortification of food products with vitamin D indicated that the key problems associated with vitamin D deficiency were solved. Over the past decade, however, two important findings have questioned this assumption. First, despite fortification of foods with vitamin D in Western countries, a considerable proportion of the population remained insufficient or even deficient in vitamin D. Certain subpopulations such as elderly individuals and patients with chronic kidney disease (CKD) are at particular risk of vitamin D deficiency. Second is the increasing awareness that vitamin D not only has a role in the regulation of bone metabolism, but also affects several other organ systems. For example, kidneys express functional vitamin D receptors (VDRs) and respond to treatment with active vitamin D. A growing number of experimental studies suggest the vitamin D axis has a renoprotective role. Maintenance of adequate serum vitamin D levels might, therefore, be of particular importance for patients with renal disease, not only for the prevention of bone disease, but also for the protection of renal function.

This Review first outlines the physiology of vitamin D and discusses the physiological processes that might be affected in patients with CKD. The epidemiology of vitamin D deficiency as well as its implications in both the general population and in patients with renal disease is also described. The main focus is on the pathogenic consequence of vitamin D deficiency in the kidney and on the renoprotective potential of pharmacological strategies that use vitamin D analogues.

Vitamin D physiology

In the presence of solar ultraviolet B radiation, vitamin D_3 is photochemically produced in the skin from 7-dehydrocholesterol (Figure 3.1). Alternatively, vitamin D_3 can be absorbed through the intestine from food sources such as oily fish, fortified dairy products and mushrooms. Ingested or photosynthesized vitamin D_3 is converted to active vitamin D (1α,25$(OH)_2$ vitamin D_3 or calcitriol, subsequently referred to in this Review as active vitamin D), via two hydroxylation steps. First, vitamin D_3 is converted by 25-hydroxylase in the liver to form 25(OH) vitamin D_3 (calcidiol). Calcidiol is subsequently converted to active vitamin D in the proximal tubular epithelial cells of the kidney. Transport of the different vitamin D metabolites to the liver, kidney, and target organs is facilitated by

the plasma vitamin D-binding protein (DBP) (2). The calcidiol-DBP complex is filtered by the glomerulus before it enters the proximal tubule cells via megalin-mediated uptake at the brush border (2). In tubular epithelial cells, the conversion of calcidiol into active vitamin D is regulated by 25(OH) vitamin D_3 1α-hydroxylase (CYP27B1). This enzyme has an important role in the regulation of circulating concentrations of active vitamin D, and is controlled by several peptide hormones and regulatory feedback loops. PTH is the main inducer of 1α-hydroxylase activity. PTH is produced by calcium-sensing cells of the parathyroid gland, mainly under hypocalcemic conditions. In addition, 1α-hydroxylase activity is induced by calcitonin (3), a hormone that is produced by the C cells of the thyroid gland under normocalcemic conditions when the PTH system is downregulated.

Circulating levels of active vitamin D are negatively regulated by several feedback mechanisms (Figure 3.1). First, vitamin D can be directly inactivated by the enzyme 1,25-dihydroxyvitamin D_3 24-hydroxylase (CYP24A1) by the addition of another hydroxyl (OH) group at position 24. This step results in the generation of 1,24,25$(OH)_3$ vitamin D_3 and ultimately produces the water-soluble calcitroic acid (1α-hydroxy-23-carboxy-24,25,-26,27-tetranorvitamin D_3), which is excreted by the kidney. Second, 1α-hydroxylase expression is downregulated by active vitamin D itself via vitamin D-responsive elements in the promoter region of 1α-hydroxylase (4). Third, active vitamin D stimulates the production of fibroblast growth factor (FGF)23 in osteocytes (5). This growth factor acts via the FGF receptor 1 (FGFR-1)-Klotho complex, which functions as an FGF23 receptor (6) to negatively regulate renal synthesis of 1α-hydroxylase (Figure 3.1) (7).

Several factors also influence circulating levels of active vitamin D. The effects of seasonal changes (8) and sunlight exposure (9) on levels of active vitamin D have been recognized for decades. Circulating vitamin D levels also differ with ethnicity, being lowest in non-Hispanic blacks (10). Diet is also a contributing factor, but the contribution of diet to overall levels of active vitamin D is limited because much higher amounts are produced in the skin (1).

The actions of active vitamin D are mediated by the VDR. This receptor is part of the nuclear receptor superfamily and is predominantly located in the nucleus, although in some cells it can be associated with caveolae in the plasma membrane. Following the recruitment of transcriptional coactivators, the activated VDR binds to vitamin D-response elements in the promoter region of target genes. An overview of the diverse VDR target genes that have been identified in several cell types has been published elsewhere (9–15).

The VDR also exerts nongenomic actions. For example, the VDR can form a complex with the p65 subunit of NFκB to produce anti-inflammatory actions (16). Another nongenomic mechanism of action of VDR is achieved by so-called rapid responses. This mechanism has been reviewed elsewhere (17). Briefly, binding of active vitamin D to caveolae-associated VDRs activates second messenger systems, which may force the opening of voltage-gated calcium or chloride channels, and facilitate cross-talk with the nucleus to modulate the expression of target genes. The VDR is expressed by more than 30 organ systems and tissues (18–20), including the kidney. Available data on the expression pattern of VDRs in the normal kidney are limited, but immunohistochemically detectable levels are expressed by proximal and distal tubular epithelial cells, glomerular parietal epithelial cells, and collecting duct cells (21). Treatment of cultured tubular epithelial cells (16), mesangial cells (22), podocytes (23), and juxtaglomerular cells (23), with ac-

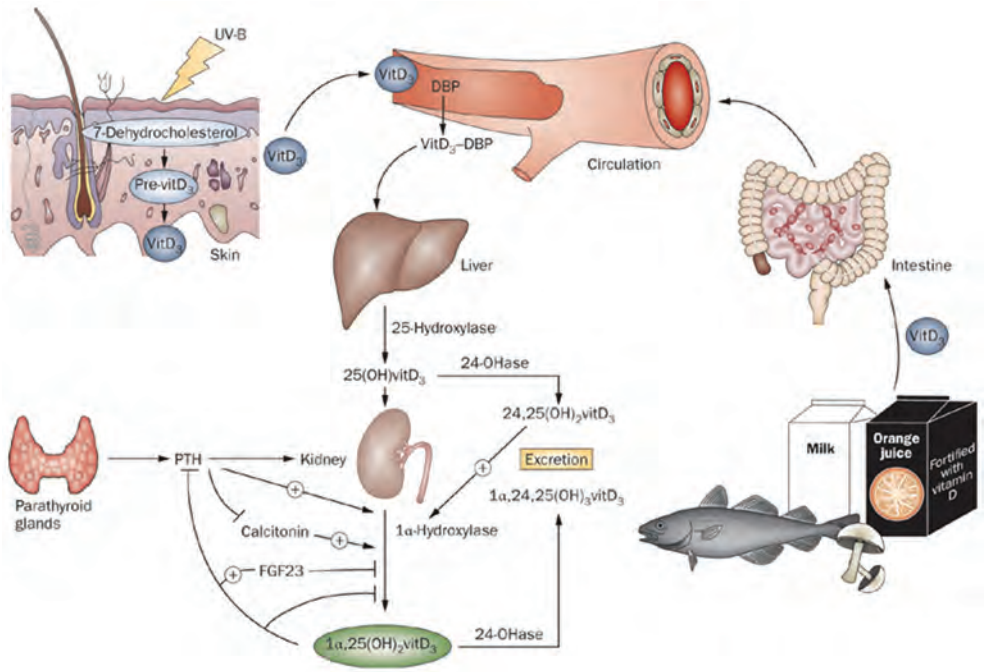

Figure 3.1: Vitamin D physiology. Vitamin D_3 is generated from 7-dehydrocholesterol in the skin under the influence of UV-B radiation or is obtained from dietary sources by absorption through the intestine. Circulating vitamin D_3 is transported in conjugation with DBP. In the liver, vitamin D_3 is converted to 25(OH) vitamin D_3 by 25-hydroxylase. 25(OH) vitamin D_3 is then converted to the active vitamin D metabolite, 1α,25(OH)$_2$ vitamin D_3 in the kidney by the enzyme 1α-hydroxylase. 1α-hydroxylase is regulated by several mechanisms, including PTH, calcitonin, and FGF23. Active vitamin D can be deactivated by addition of another hydroxyl (OH) group at the position 24, after which the end product is excreted. Abbreviations: 24-OHase, 1,25-dihydroxyvitamin D_3 24-hydroxylase; DBP, vitamin D-binding protein; FGF, fibroblast growth factor; PTH, parathyroid hormone; UV, ultraviolet; vit, vitamin. Permission obtained from Nature Publishing Group Ltd © Deeb, K. K. *et al.* Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nat. Rev. Cancer* 7, 684-700 (2007).

tive vitamin D or vitamin D analogues such as paricalcitol, modulated the expression of target genes, suggesting the presence of a functional VDR in these cells. Whether renal VDR expression is modulated in patients with CKD, is unknown.

Epidemiology of vitamin D deficiency

The definition of vitamin D deficiency is still a matter of debate. Some consider serum levels of 25(OH) vitamin D_3 < 75 nmol/l (< 30 ng/ml) to be insufficient, levels < 37 nmol/l (< 15 ng/ml) deficient, and levels < 25 nmol/l (< 10 ng/ml) severely deficient (24). However, most experts define vitamin D deficiency as 25(OH) vitamin D_3 levels < 50 nmol/l (< 20 ng/ml) (1, 25–27). A review of several reports of vitamin D intoxication suggests that serum levels of 25(OH) vitamin D_3 > 375-500 nmol/l (> 150-200 ng/ml) are associated with a risk of hypercalcemia and should be considered toxic (1, 28).

Data from consecutive cohort studies of the general population from the National Health and Nutrition Examination Survey (NHANES) suggested that changes had occurred in levels of serum vitamin D in the US population between the periods of 1988-1994 (NHANES III) and 2001-2004 (10). This change might be related to the increased use of sunscreen (29), decreased amounts of outdoor activity (30), and the increased prevalence of obesity (31), all of which are associated with reduced vitamin D levels. Although these data raise considerable concerns, the validity of comparing different measurements of 25(OH) vitamin D_3 has been questioned because of methodological issues that can arise due to calibration problems, lack of standard operating procedures and the use of a variety of equipment (32–34). Adjusting for methodological changes in the measurement of vitamin D levels demonstrated that much of the observed difference in vitamin D levels between the NHANES III and NHANES 2001-2004 studies could be accounted for by changes unrelated to actual changes in vitamin D status (35). Until a reference measurement procedure for 25(OH) vitamin D_3 is available, caution must be taken with the interpretation of epidemiological data on vitamin D (36).

Patients with CKD, in particular those on dialysis, generally have more severe vitamin D deficiency than the general population (10, 37–39). Vitamin D insufficiency (defined as levels < 75 nmol/l [< 30 ng/ml]) and severe vitamin D deficiency (defined as levels < 25 nmol/l [< 10 ng/ml]) are both more common in patients with predialysis CKD than in the normal population (Figure 3.2) (37). Some, but not all, studies have found a positive association between stage of CKD and prevalence of vitamin D deficiency (40, 41). Predialysis CKD patients who live in a sunny country are less likely to be vitamin D insufficient or deficient than those who live in less sunny areas (42). In a cross-sectional cohort of 825 dialysis patients in the US, only 22% had 25(OH) vitamin D_3 levels of > 75 nmol/l (> 30 ng/ml), while 60% had levels between 25 and 75 nmol/l (10 and 30 ng/ml) (43). In a French study of dialysis patients, 42% had severe vitamin D deficiency (Figure 3.2) (38). An analysis of patients on peritoneal dialysis showed similar percentages (44). Thus, in patients with end-stage renal disease, vitamin D deficiency is an even more prevalent and severe problem than it is in patients with mild to moderate renal insufficiency.

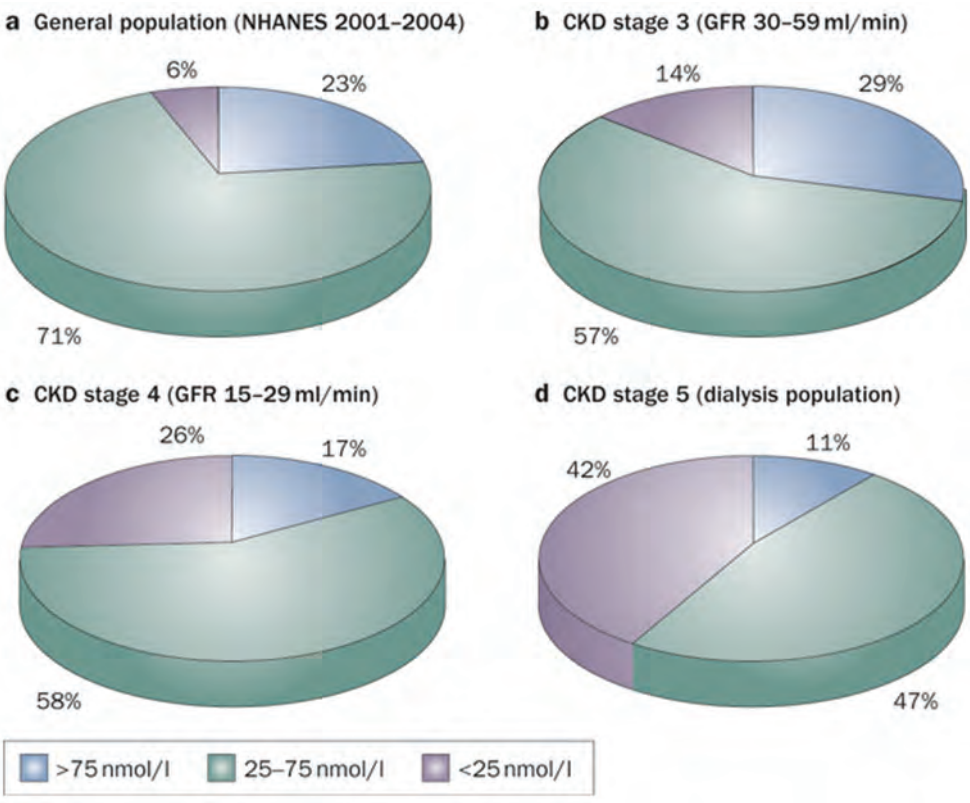

Figure 3.2: Prevalence of vitamin D deficiency in the general population and in patients with chronic kidney disease. Prevalence of vitamin D levels > 75 nmol/l (> 30 ng/ml) (defined by some researchers as sufficient), between 25 and 75 nmol/l (10 and 30 ng/ml), and < 25 nmol/l (< 10 ng/ml) (severely deficient) in the a — general population (8), b,c — in patients with preterminal chronic kidney disease (stages 3 and 4) (37), and d — hemodialysis patients (stage 5) (38). Vitamin D insufficiency is common in all categories of patients, whereas severe deficiency seems to be more prevalent in those with more severe renal disease. Abbreviations: CKD, chronic kidney disease; GFR, glomerular filtration rate.

Implications of vitamin D deficiency

In the general population, vitamin D deficiency has been independently associated with a small but significantly increased risk of all-cause mortality (45–47). Other studies found that vitamin D deficiency may be associated with cardiovascular morbidity (48) and mortality (49). A subgroup analysis showed that the strongest associations between vitamin D deficiency and mortality occurred in patients without cardiovascular disease, hypertension, or diabetes. This finding may imply that vitamin D deficiency exerts detrimental actions before cardiovascular disease is established. Whether vitamin D treatment could provide cardiovascular protection has been the subject of intense debate since the publication of negative findings by Hsia *et al.* (50) in 2007. Of note, researchers have commented that the vitamin D replacement dose used in that study may have been inadequate to achieve an effect (51).

The relationship between vitamin D deficiency and impaired bone metabolism is illustrated by the role of vitamin D deficiency in the development of rickets and osteomalacia (52). Several studies have documented beneficial effects of vitamin D supplementation ($\geq$700-800 IU daily in combination with calcium) in patients with osteoporosis (53–56). Lower doses of vitamin D_3, or doses that do not lead to the restoration of adequate serum 25(OH) vitamin D_3 levels, may not effectively lower fracture risk (25, 57).

In the NHANES III cohort, vitamin D deficiency was associated with an increased risk of albuminuria (58). Whether vitamin D deficiency is the cause or consequence (through reduced reuptake of the calcidiol-DBP complex in tubular cells under proteinuric conditions, as is discussed below) of albuminuria, or whether it is an epiphenomenon, is the subject of ongoing randomized, controlled trials (59). In patients with CKD, several studies have demonstrated an independent inverse association between serum levels of active vitamin D and the rate of renal function loss or mortality (41, 60, 61). Similarly, Wolf *et al.* reported an association between vitamin D deficiency and early mortality in a cohort of patients on dialysis (43). Importantly, subsequent treatment of these patients with active vitamin D was associated with increased survival.

Low serum vitamin D levels are more strongly associated with increased risk of coronary atherosclerosis in patients with CKD than in individuals with normal renal function (62). In a population of patients on peritoneal dialysis, the risk of cardiovascular morbidity was higher in individuals with vitamin D deficiency than in those without (44). In other studies, vitamin D deficiency was independently associated with several cardiovascular risk factors such as hypertension, insulin resistance, diabetes, and dyslipidemia (63, 64). These associations could at least in part explain the increased incidence of cardiovascular morbidity and mortality in patients with CKD and vitamin D deficiency. Osteoporosis and osteopenia are common in patients with CKD (65, 66). This detrimental effect on bone is most likely related to disturbed vitamin D and PTH metabolism in these patients.

The prevalence of vitamin D deficiency in patients with CKD and the association of vitamin D deficiency with increased mortality, cardiovascular morbidity and bone disease, warrants adequate correction of vitamin D levels well before these patients develop end-stage renal disease. The Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines advise vitamin D supplementation in patients with stages 3-4 CKD if serum 25(OH) vitamin D_3 level is $<$ 75 nmol/l ($<$ 30 ng/ml) to reduce the risk of low bone mineral density and hip fracture (67). Increasing understanding of the potential renoprotective

effects of active vitamin D might provide an additional reason for the early initiation of vitamin D supplementation in patients with renal disease. Emerging data suggests that correction of vitamin D may potentially slow the progression of renal disease in these patients.

Vitamin D deficiency in renal disease

Mechanisms of reduced vitamin D levels

After filtration by the glomerulus, the reuptake of 25(OH) vitamin D_3 (bound to DBP), takes place at the brush border of the proximal tubular epithelial cells (Figure 3.3). This process is mediated by megalin, and can result in release of either active 1,25$(OH)_2$ vitamin D_3 or inactive 25(OH) vitamin D_3 into the circulation on the basolateral side. Although reduced renal megalin expression has not been demonstrated in patients with renal disease, studies in cultured tubular epithelial cells suggest that chronic exposure to albumin reduces megalin expression (68). In addition, renal megalin messenger RNA (mRNA) expression is reduced in models of experimental nephrotic syndrome *in vivo* (69). Furthermore, megalin knockout mice develop low-molecular-weight proteinuria and actually lose large amounts of vitamin D-DBP in their urine (70). These findings are supported by studies in a kidney-specific megalin knockout mouse-when fed a vitamin D-depleted diet, these mice demonstrated lower circulating levels of 25(OH) vitamin D_3 and strongly elevated 25(OH) vitamin D_3-DBP excretion than did wildtype mice (71). Furthermore, kidney-specific megalin knockout mice were hypocalcemic and had osteomalacia (softening of the bones) (71). In a rat model of nephrotic syndrome induced by puromycin aminonucleoside, serum levels of 25(OH) vitamin D_3 decreased concomitantly with increased proteinuria (72). Since liver mRNA expression of both CYP2R1 (the protein product of which metabolizes vitamin D_3 to 25(OH) vitamin D_3) and the gene that encodes DBP were unchanged, the decreased levels of 25(OH) vitamin D_3 might not be a result of dysfunctional hydroxylation or transport to the liver. Rather, these studies indicate that 25(OH) vitamin D_3-DBP might be lost into the urine in the nephrotic state. Alternatively, the reduced serum levels of 25(OH) vitamin D_3 in CKD patients might occur secondary to decreased photoproduction of pre-vitamin D_3 from 7-dehydrocholesterol in the skin because of uremia (73).

Reduced activity of 1α-hydroxylase is widely accepted to be the main cause of reduced circulating levels of 1,25$(OH)_2$ vitamin D_3 in patients with CKD (74). In a model of experimental nephrosis, renal mRNA expression of 1-hydroxylase is decreased and CYP24A1 is increased 3 days after induction of nephrosis, resulting in a reduction in serum levels of 1,25$(OH)_2$ vitamin D_3 (72). Therefore, in patients with CKD, vitamin D deficiency probably results from multiple factors including urinary loss of 25(OH) vitamin D_3-DBP associated with proteinuria (72), reduced activity of 1α-hydroxylase, and compromised endogenous pre-vitamin D_3 production in the skin (73).

Renoprotective potential of vitamin D

Growing evidence indicates that vitamin D analogues may have beneficial effects in patients with CKD. Antifibrotic effects of vitamin D analogues have been demonstrated in

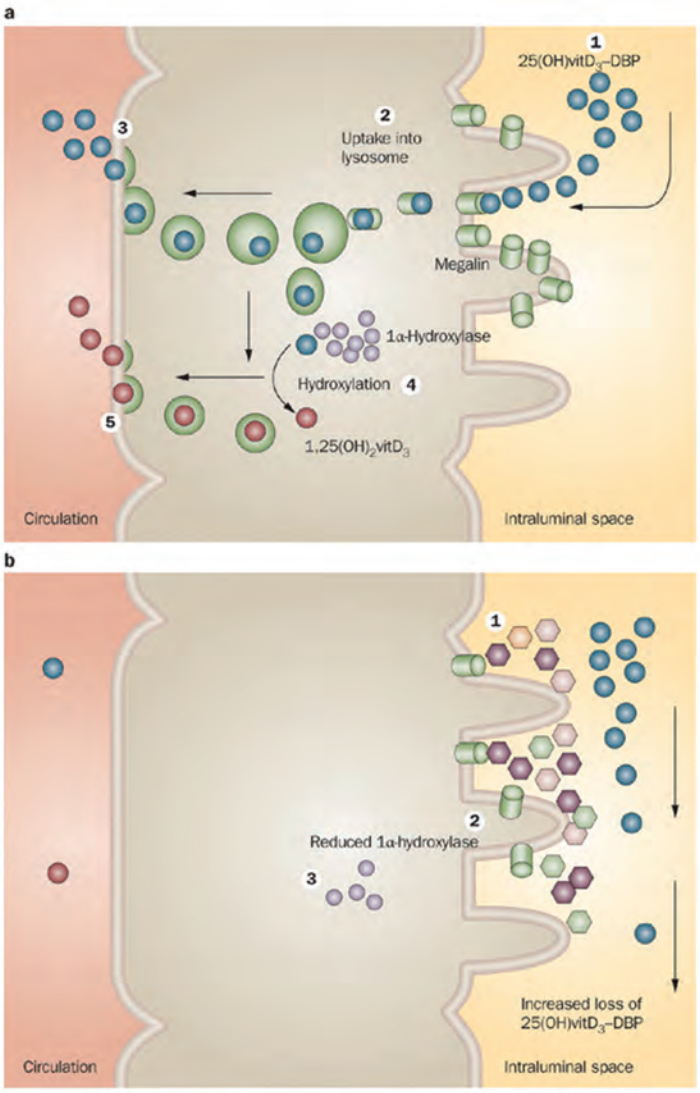

Figure 3.3: Possible mechanisms of vitamin D deficiency in proteinuric renal disease. a — In healthy individuals, 25(OH) vitamin D_3 bound to DBP is filtered through the glomerulus (1). In proximal tubular cells, uptake of 25(OH) vitamin D_3-DBP is facilitated by megalin. After uptake into lysosomes (2), 25(OH) vitamin D_3-DBP is either returned to the circulation (3), or converted to active vitamin D by hydroxylation by 1-hydroxylase (4), and released into the circulation (5). b — In patients with proteinuria, megalin is occupied by an extensive protein load that consists mainly of albumin (1); thus, fewer receptors are available to reabsorb 25(OH) vitamin D_3-DBP. In addition, as a consequence of proteinuria, proximal tubular cells are damaged and express less megalin (2), which also reduces 25(OH) vitamin D_3-DBP reabsorption. 1-hydroxylase expression is also reduced in patients with chronic kidney disease (3), which results in decreased formation of active vitamin D. Abbreviation: DBP, vitamin D-binding protein; vit, vitamin.

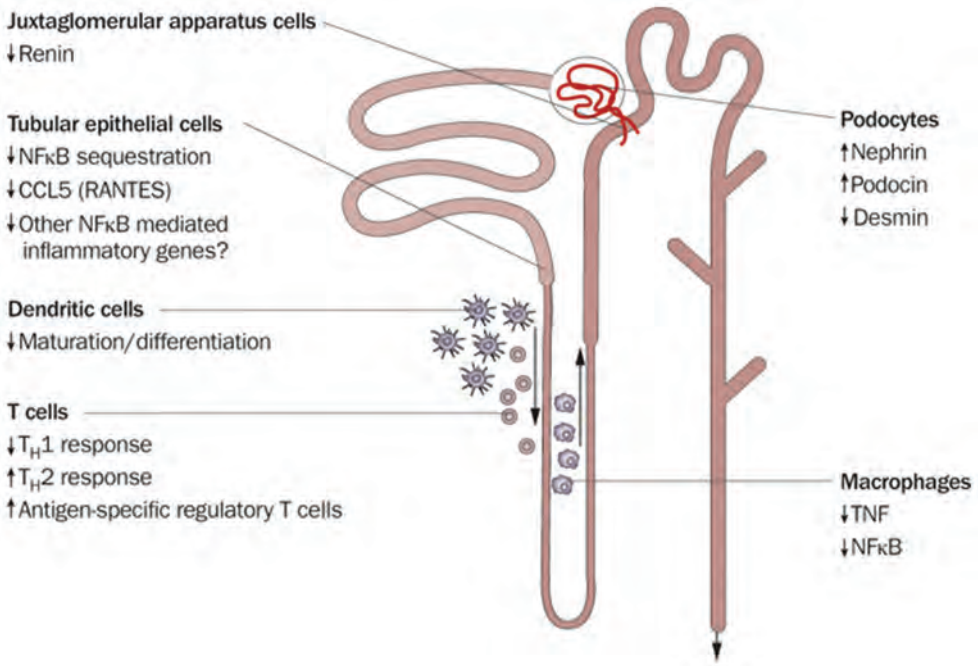

Figure 3.4: Overview of the potential renoprotective effects of active vitamin D in the damaged kidney. In the glomerulus, vitamin D protects podocytes by increasing the expression of nephrin and podocin. Under nephrotic conditions, vitamin D reduces the expression of desmin, a marker of podocyte injury. In juxtaglomerular apparatus cells, renin production is inhibited by vitamin D. In macrophages, vitamin D reduces TNF and NFκB expression. Similarly, vitamin D has anti-inflammatory effects in injured tubular epithelial cells, by reducing NFκB availability. Consequently, expression of chemokines such as CCL5 (RANTES), are reduced. Vitamin D can also modify T-helper-cell responses and affect the maturation and differentiation of dendritic cells, which provides protection against inflammatory damage in response to renal injury. Abbreviations: CCL5, CC-chemokine ligand 5; NFκB, nuclear factor κB; T_H, T-helper; TNF, tumor necrosis factor.

cultured renal cells (75) and in animal models of renal damage (76). Several mechanisms could be involved in this renoprotective effect, including direct antiproteinuric effects through the protection of podocytes, interactions with the renin-angiotensin-aldosterone system (RAAS), and anti-inflammatory effects (Figure 3.4).

Antiproteinuric effects

In experimental puromycin aminonucleoside-induced nephrotic syndrome, treatment with active vitamin D or the vitamin D analogue 22-oxacalcitriol has demonstrated clear antiproteinuric effects (72, 77). Administration of active vitamin D or 22-oxacalcitriol partially restored glomerular expression of nephrin, an important structural slit diaphragm protein that is inversely correlated with proteinuria. In podocytes, active vitamin D induces nephrin gene expression (possibly in cooperation with the retinoic acid receptor (78)), probably by binding of the activated VDR to vitamin D response elements in the nephrin gene (79). In addition, vitamin D treatment restores expression of podocin, a molecule associated with the slit diaphragm, and decreases expression of the podocyte injury marker desmin (72). Together, these findings indicate that active vitamin D has a direct protective effect on podocytes, and possibly explains the antiproteinuric effects of vitamin D that have been reported by other studies (80, 81). As proteinuria is a marker of progressive renal dysfunction, these data support the potential of vitamin D administration for long-term renoprotection. Studies to assess the antiproteinuric effects of vitamin D analogues in humans are currently underway. As RAAS blockade is first method of choice for reducing proteinuria, the effects of vitamin D administration together with RAAS blockade are also under investigation (59).

RAAS-related effects

The antiproteinuric effects of vitamin D and its analogues could, at least in part, be explained by actions on the RAAS. Two key publications demonstrated that active vitamin D binds to the promoter region of the renin gene to downregulate renal mRNA expression of renin (68, 82). In VDR knockout mice, inhibition of renin transcription by vitamin D is reduced, which results in RAAS activation and renal injury (23, 82). VDR knockout mice are hypertensive, probably as a consequence of RAAS activation (82). Whether this finding implies that administration of vitamin D analogues could have antihypertensive effects is currently a subject of controversy (83). In cultured juxtaglomerular-like cells, the administration of active vitamin D reduces renin expression by 90% (82) by blocking the cyclic adenosine monophosphate response element in the renin gene promoter (68) In adipocytes, 1,25$(OH)_2$ vitamin D_3 dose-dependently decreases expression of the gene that encodes the type I angiotensin II receptor (84). Whether VDR and type I angiotensin II receptor interact in renal cells is unknown.

RAAS blockade is currently first-line renoprotection therapy for patients with CKD, but can be associated with a reactive rise in renin (85, 86). Both angiotensin-converting-enzyme (ACE) inhibitors and type I angiotensin II antagonists require concomitant adherence to a low sodium diet and administration of a diuretic to achieve optimal effects on blood pressure and proteinuria (87, 88); however, the benefits of these approaches come at the expense of a potentiated reactive rise in levels of both renin and aldosterone. Data

support direct profibrotic effects of aldosterone (89) and renin by direct effects on the (pro)-renin receptor (90). Thus, the eventual therapeutic benefit of volume depletion by RAAS blockade can be limited by the reactive rise in renin levels. Data from studies in rats support the presence of profibrotic renal effects associated with aggressive volume depletion under RAAS blockade (91). These data, therefore, suggest that blocking the reactive rise in renin during RAAS blockade could have therapeutic benefits. In our opinion, vitamin D is a potential candidate for this purpose. The addition of vitamin D to RAAS blockade therapy might improve the efficacy of RAAS blockade by blunting the reactive rise in renin (and the downstream rise in aldosterone). Indeed, treatment with a combination of paricalcitol and enalapril reduced renal damage in the 5/6 nephrectomy rat model more strongly than did paricalcitol or enalapril alone (92).

Direct inhibition of renin has long been known to have renoprotective potential (93) and may suppress the reactive rise in renin activity. Studies over the past few years have investigated the renoprotective effects achieved by combined treatment with conventional RAAS blockers (that is, ACE inhibitors and angiotensin II receptor blockers) and the direct renin inhibitor aliskiren (94–96). In patients with type 2 diabetes, hypertension, and diabetic nephropathy, combination treatment with aliskiren and losartan for 6 months reduced proteinuria to a greater extent than did losartan alone (94). The reduction in proteinuria seemed to occur independently of blood pressure, which was not significantly affected by treatment. However, the long-term renoprotective effects of combined renin and ACE inhibition or angiotensin II receptor blockade remain to be documented. For the purpose of the current Review, it should be mentioned that direct renin inhibitors affect the enzymatic activity of renin but not its production or its interaction with the prorenin receptor (97). Therefore, inhibition of renin production by vitamin D may still have therapeutic potential for patients with CKD (98).

The anti-inflammatory properties of vitamin D might also add to the value of vitamin D as an adjunct to RAAS blockade, as inflammation is a determinant of RAAS blockade resistance (99, 100). Vitamin D therapy could be particularly beneficial in patients for whom additional RAAS blockade therapy is contraindicated, such as those with hypotension or hyperkalemia.

Anti-inflammatory and immunomodulatory effects

Several studies have demonstrated the anti-inflammatory properties of active vitamin D (16, 22, 101). The concept of vitamin D as an immunomodulatory molecule originated over 25 years ago (102, 103), but the mechanisms involved, and the relevance of these effects to the treatment of renal disease, have only now begun to be understood (104). A study by Zehnder *et al.* reported that serum $1,25(OH)_2$ vitamin D_3 levels were inversely associated with renal inflammation in several types of kidney disease (105). As mentioned previously, treatment of cultured proximal tubular epithelial cells with the vitamin D analogue paricalcitol induced the formation of a complex that consisted of VDR and the NFκB component p65 (16). The formation of this complex led to the reduced binding of NFκB to the promoter regions of genes involved in the inflammatory response, resulting in decreased activation of genes such as CCL5 (also known as RANTES). Similar results were achieved in mesangial cells-administration of active vitamin D inhibited the activity of CCL2 (also known as MCP-1) by stabilization of the NFκB inhibitory unit IκBα (22).

In macrophages, active vitamin D also suppressed NFκB activity, leading to reduced production of TNF (106). These findings emphasize that VDR regulates the expression of target genes both directly, and also by altering the activity of transcription factors such as NFκB.

Of interest, in addition to processing in the liver and the kidneys, vitamin D can also be metabolized by cells of the immune system (107). Specifically, activated T cells can convert 25(OH) vitamin D_3 to active vitamin D (107). Moreover, macrophages and some dendritic cells express enzymes required to convert vitamin D_3 into active vitamin D (107–109). The net effect of active vitamin D on T cells is to block the production of cytokines, in particular interferon γ, by T-helper-1 cells, and to promote T-helper-2 cell responses (110). Vitamin D also has inhibitory effects on dendritic cell maturation and differentiation *in vitro*, which results in a marked T-cell hyporesponsiveness (that is, reduced T-cell proliferation and interferon γ production) (111). Vitamin D may have an important role in the maintenance of peripheral tolerance by expanding the pool of antigen-specific regulatory T cells (112, 113). Together, these findings justify studies that investigate the potential of vitamin D analogues for the treatment of inflammatory kidney diseases such as autoimmune nephritis, and for tolerance induction in renal transplant recipients. Preclinical studies in rats have shown improved graft survival following treatment with active vitamin D after kidney transplantation (114). Although cholecalciferol treatment (100,000 IU every 2 weeks for 2 months) in vitamin D-deficient kidney transplant patients has been shown to be safe (115), the dose required to induce renal allograft tolerance or to prolong allograft or patient survival is not known.

Vitamin D could also have important immunomodulatory effects in individuals with non-autoimmune chronic renal disease and in those who do not yet require kidney transplantation. Of note, a recent study demonstrated the importance of renal dendritic cells in the progression of glomerulonephritis after glomerular injury (116). Vitamin D-mediated inhibition of dendritic cell maturation and differentiation might reduce the inflammatory response associated with glomerular injury, which might slow or potentially even halt progression of renal damage.

Conclusions

The kidney has a key role in the metabolism of vitamin D; consequently, renal disease affects vitamin D metabolism. Epidemiological studies indicate that the prevalence of vitamin D deficiency in the general population is considerable, but is higher in patients with CKD. This latter finding is important since low circulating levels of vitamin D have been associated with increased morbidity and mortality, particularly in patients with CKD; however, whether a causal relationship exists remains to be determined.

In addition to the classic effects of vitamin D on calcium and phosphate homeostasis, several studies have revealed other important functions of this hormone. For example, administration of vitamin D in experimental CKD reduces proteinuria, which may relate to the effects of vitamin D on the RAAS and anti-inflammatory actions. Vitamin D decreases RAAS activity by downregulation of renin gene transcription in juxtaglomerular cells. The inhibitory effect on renin is of particular interest, given the reactive rise in renin during RAAS blockade, the cornerstone of current pharmacological treatment in patients

with CKD. Vitamin D might, therefore, be a suitable add-on strategy to RAAS blockade in these patients. Long-term, randomized, controlled trials should address the renoprotective potential of this approach.

The relationship between vitamin D and the immune system is currently under intense investigation. The role of vitamin D in macrophages, T cells and dendritic cells is of particular interest, since these cells have a substantial role in the pathophysiology of renal damage. Results from clinical trials that have used vitamin D analogues in patients with CKD (59) and renal transplant recipients (117) are expected in the near future.

In conclusion, emerging data indicate that vitamin D deficiency has greater clinical consequences than the induction of osteomalacia in non-renal and renal populations. Although the prevalence of rickets has decreased in Western countries, other disease entities that are related to vitamin D deficiency have been recognized. Clinical trials to investigate the role of vitamin D supplementation for renal and cardiovascular protection are currently underway, and will enable the role of vitamin D supplementation in the management of CKD to be better defined.

Acknowledgments

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